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Determining the Origin of Fe3+-induced BODIPY Probe Fluorescence in Aqueous Solution

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Determining the Origin of Fe^{3+}-induced BODIPY Probe Fluorescence in Aqueous Solution

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By:

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Project Advisor

Abstract

Iron is the most physiologically abundant transition metal with numerous applications to bodily systems and disease states. Too little iron will affect oxygen metabolism, a critical function of free iron, while excess iron has been associated with diseases including hepatitis, hemochromatosis, and neurological degeneration. The medical importance of iron homeostasis warrants research into developing accurate aqueous chemosensors. Sensors created with BODIPY fluorophores have been studied heavily in recent years for this purpose. Many published studies of these sensors identify iron binding to the probe as the source of the fluorescent signal. The purpose of this project was to synthesize and characterize two BODIPY iron sensors to demonstrate Fe^{3+} binding is not necessary to induce a fluorescence response. Fe^{3+} precipitates in water to produce iron (III) hydroxide, indicating that the fluorescence seen in published literature may not be caused by iron binding to the receptor of BODIPY. The compounds created in this MQP do not have a strong binding affinity, therefore it was hypothesized that if the molecule still fluoresces, this is due to other causes and can be used comparatively as a control when studying BODIPY based molecules that have strong Fe^{3+} recognition units.

Acknowledgements

I would like thank the Burdette research lab group for their support and guidance of this MQP: Shawn Burdette, Prem Basa, Chelsea Barr and Jingjing Yan. I would also like to thank WPI for its commitment to undergraduate research.

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1. Introduction

Iron is the most physiologically abundant transition metal and has roles in numerous cellular functions.[1] Humans as well as other mammals and even bacteria require iron for biological processes including oxygen metabolism, electron transfer, transcription and reproduction.[2-5] Due to iron's abundance and versatility in the body, it also plays a role in many disease states. Levels of iron are normally maintained by homeostasis, however, iron concentration can fluctuate in the body resulting in illness. Too little iron in the body can weaken the immune system and cause blood clotting disorders, while excess iron in the body has been associated with the development of cancer, hepatitis and hemochromatosis.[6, 7] Additionally, much effort has been recently devoted to the study of iron's potential roles in neurodegenerative diseases such as Parkinson's and Alzheimer's.[8-11]

The clinical significance of iron homeostasis warrants research into developing accurate chemosensors which can detect Fe^{3+} under aqueous conditions. A chemosensor is a molecule which contains a receptor site that binds to the target analyte, in this case Fe^{3+}, resulting in a response from the signaling unit of the molecule. One molecule which is particularly useful for this application is BODIPY, a fluorophore which has been researched extensively in recent years.[12, 13] Some published research cites studies of these sensors in methanol which then extrapolate the results to water-based systems.

The Burdette research group has synthesized several BODIPY-based sensors, and this MQP is a continuation of that research.[1, 14] More specifically, this project hypothesized that these BODIPY-based sensors for iron may not be working as reported in aqueous solution due to the fact that Fe^{3+} will precipitate in water to form iron (III) hydroxide. To study this, two BODIPY-

based sensors with a weak binding affinity for iron were synthesized. Both hydroxy and methoxy BODIPY derivatives were synthesized and characterized by ^{1}H NMR, ^{13}C NMR, LC-MS, and FT-IR. In future research, these sensors will be studied using UV-vis and fluorescence studies and compared to the published fluorescence data of iron-specific BODIPY sensors.

2. Background

It is important to understand the clinical significance of iron homeostasis, its biological abundance and why we need to research how to accurately detect levels of iron in solution. BODIPY-based iron sensors will be introduced as a way to detect iron in organic solutions, and potential downfalls of these sensors in aqueous solution will be discussed.

2.1 Clinical Significance of Iron Metal Ions

Iron is the most physiologically abundant transition metal and has roles in numerous biological processes including oxygen metabolism, electron transfer, transcription and reproduction.[1-5] Research into iron homeostasis has uncovered that iron imbalances in the body play roles in many different diseases including anemia, blood clotting disorders, hepatitis, hemochromatosis and some cancers.[6,7] Additionally, recent research has suggested that there may be a link between iron and neurodegenerative diseases such as Parkinson's and Alzheimer's [8-11]. It is thought that ferrous iron reacts with hydrogen peroxide via the Fenton Reaction to form hydroxide and also hydroxyl free radicals (•OH).[15] These hydroxyl free radicals are very reactive and suspected to play a role in the development and progression of neurodegeneration.[16-18] Due to iron's abundance and complex roles in biological processes and disease states, it warrants research into developing accurate chemosensors which can function in aqueous solution.

2.2 Chemosensors

A chemosensor is a molecule which contains a signaling unit, spacer, and receptor site. The target being sensed, in this case Fe^{3+}, is known as the analyte. When the analyte binds to the specific receptor site, the signaling unit responds. In the case of this MQP, the signaling unit responds by fluorescing.

Figure 1: Chemosensor Mechanics. A receptor site binds to the desired analyte resulting in a response from the signaling unit. This response results in the molecule "sensing" the presence of the analyte.

2.2 BODIPY Fluorophores

BODIPY, also known as boron dipyrromethene, is an organic dye which possesses many unique photophysical properties. Due to this, it is a great fluorophore to be used as the signaling unit in a chemosensor. When iron binds to the receptor unit of the molecule, it allows for electron flow through the iron which, in turn, results in the visible BODIPY fluorescence.

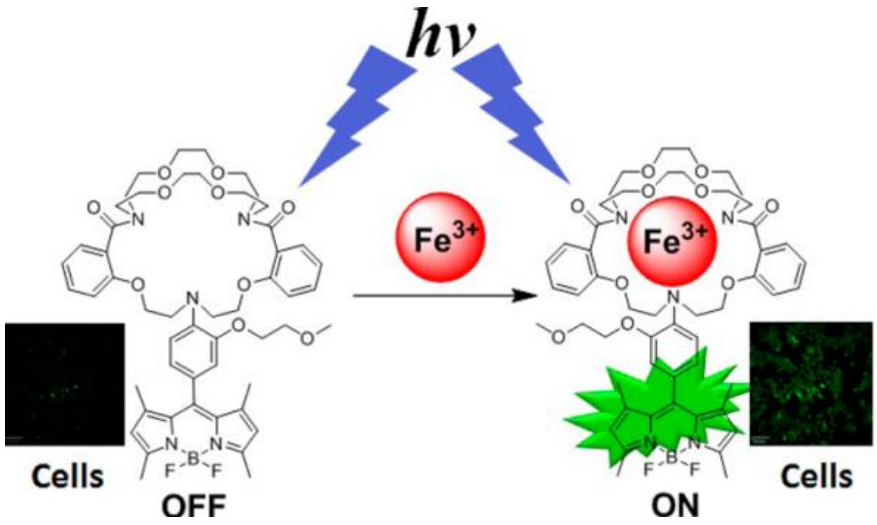


Figure 2: BODIPY-based Chemosensor Example. The sensor without Fe^{3+} present induces minimal fluorescence response, however, upon addition of the analyte, the sensor binds to iron and the signaling unit fluoresces.[19]

2.3 Existing Research

A published study of BODIPY-based iron sensors was used to make hypotheses which partially formed the basis of this MQP. The study in question used a BODIPY sensor which was responsive to iron to demonstrate the ability for the molecule to act as a turn-on fluorescence probe.[19]

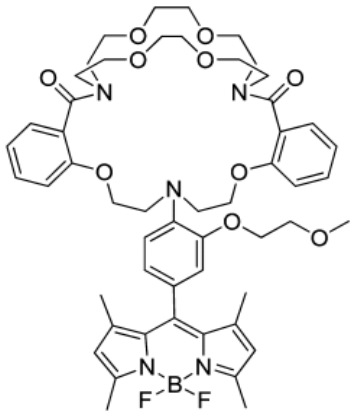


Figure 3: Iron-specific BODIPY sensor. This molecule has a strong binding affinity for iron when compared to the molecules synthesized in this MQP, therefore it can be used comparatively to assess differences in fluorescence.[19]

This sensor was then used in cells to show the effect on fluorescence in cells with just sensor and with sensor and iron present.[19]

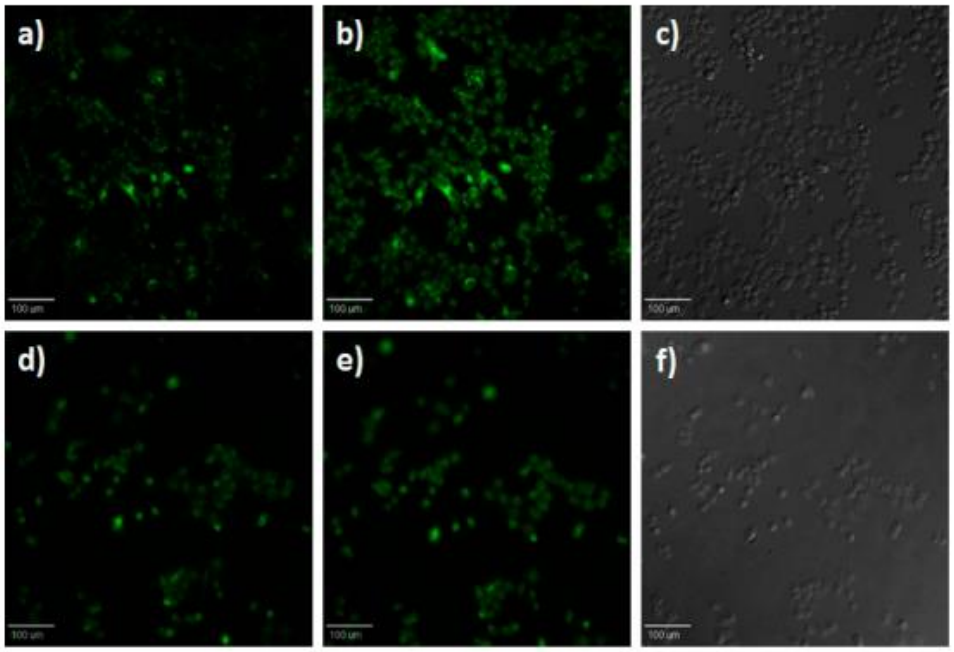


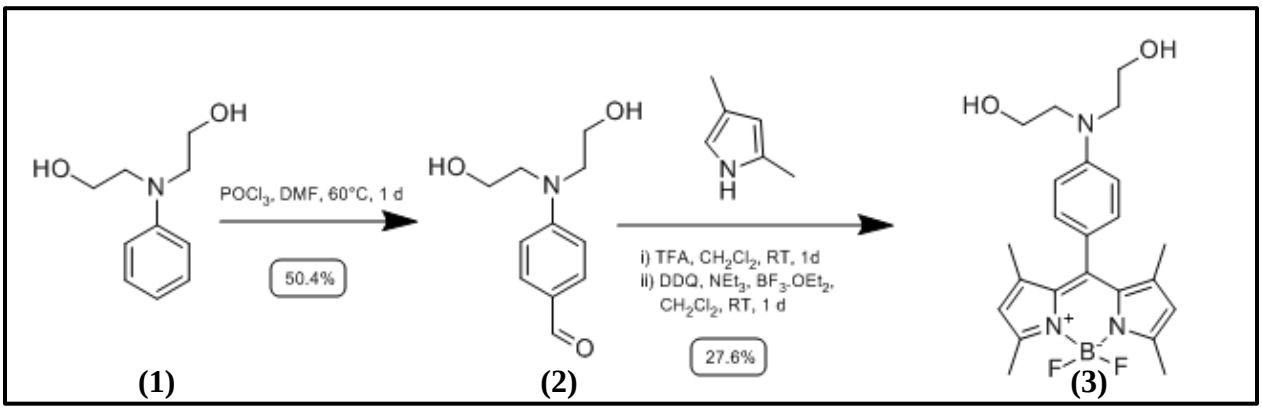
Figure 4: Cellular Imaging of BODIPY Sensor Florescence Function. The top row represents cells treated with sensor and iron, while the bottom row represents cells treated with just sensor. The difference from column 1 to column 2 is 1hr incubation time.[19]

This figure provoked further inquiry into understanding how the iron probes were analyzed in cells. The top row of cells visually appeared to be of higher confluency, while the bottom row of cells appeared visually blurry. The fluorescence displayed in the bottom row of cells, meant to act as a control of just sensor, indicates that iron binding may potentially not be required for these BODIPY-based sensors to fluoresce. Fluorescence may be caused by something else. Determining the true cause of BODIPY-sensor florescence is the long-term objective of this project.

3. Methodology

3.1 General Synthetic Procedures

All materials were obtained in the highest purity available from Fisher, Acros Organic and Alfa Aesar and used without further purification. Solvents were purged with argon and dried using a Seca Solvent Purification System. ^{1}H and ^{13}C NMR were recorded using a 500 MHz Bruker-Biospin NMR instrument, and chemical shifts are reported in ppm on the δ scale relative to tetramethylsilane (TMS). FT-IR spectra were recorded using Bruker Optics Vertex 70 with MIR source as neat crystalline powdered samples and Spectrum 100 Version 10.4.2 (PerkinElmer) fitted with diamond ATR as oils. Melting-point information was obtained using Hydrothermal Mel-Temp instrument. Full syntheses are shown in Scheme 1 and Scheme 2.



Scheme 1: Hydroxy Compound Synthesis

3.2 Hydroxy Aldehyde

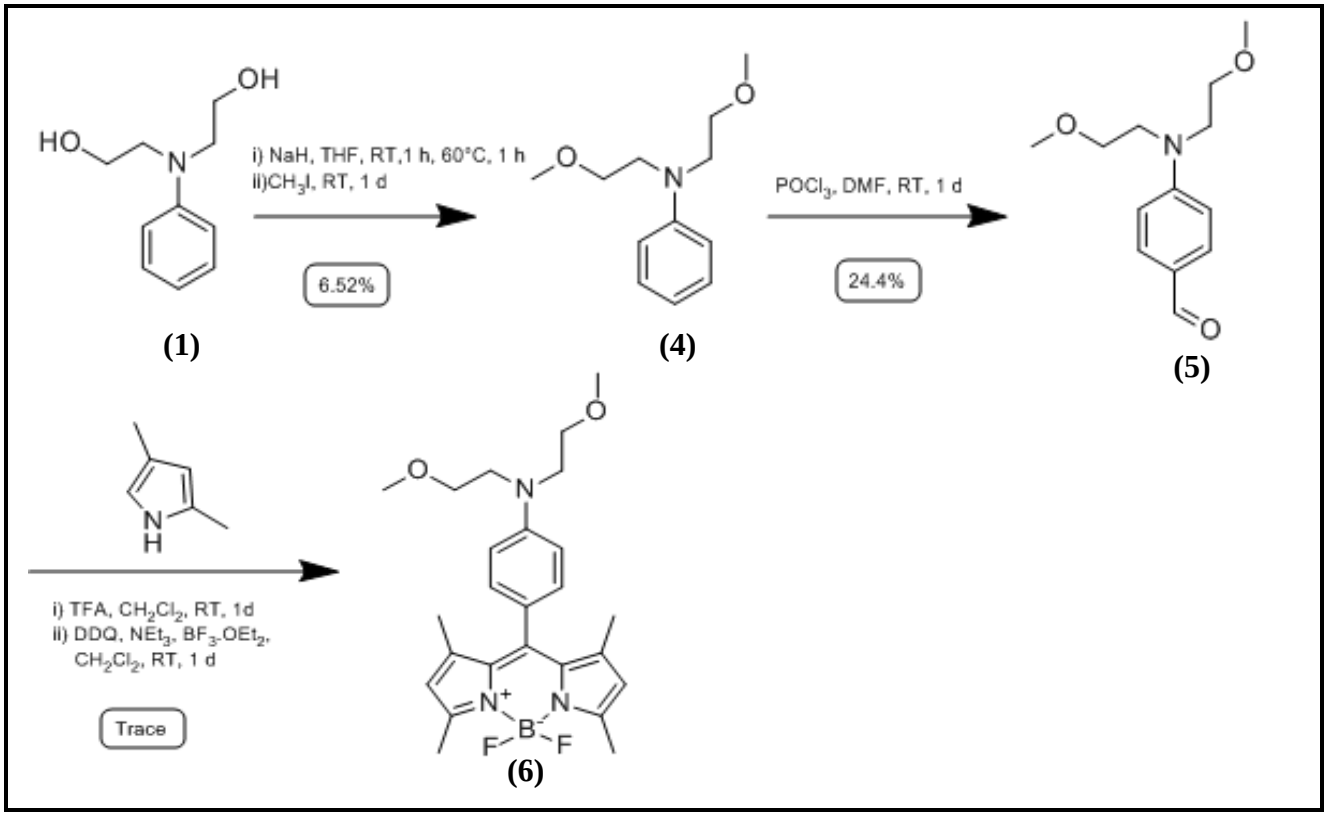
4-[bis(2-hydroxyethyl)amino]benzaldehyde (2) N-phenyldiethanolamine (2.0 g, 11.0 mmol) was combined with phosphorus oxychloride (8.43 g, 55.0 mmol) in DMF (20 mL). After 24 h in a 60 ° C oil bath, the reaction mixture was applied over ice and neutralized to pH 7 with saturated $NaHCO_3$. The product was extracted using saturated NaCl solution and ethyl acetate (3

× 50 mL). The combined extracts were dried over $NaSO_4$. Solvent removal under reduced pressure yielded yellow oil. The resultant oil was purified using column chromatography with 1:1 hexanes: ether as the eluent and yielded a white solid (1.17 g, 50.4 % yield); mp =80-82 ° C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.79 (s, 1 H), 7.79 (d, J = 9.0 Hz, 2 H), 6.76 (d, J = 9.0 Hz, 2 H), 3.86 (t, J = 7.0 Hz, 4 H), 3.70 (t, J = 7.0 Hz, 4 H). 13C NMR (500 MHz, $CDCl_3$) δ 190.4, 151.2, 132.5, 111.5, 53.5, 40.2. FT-IR (neat, cm-1) 2963.8, 2822.2, 2747.0, 2324.5, 2163.9, 1665.8, 1588.9, 1559.9, 1521.2, 1461.1, 1433.0, 1403.0, 1360.9, 1313.0, 1284.2, 1254.3, 1240.9, 1215.6, 1194.1, 1163.2, 1124.3, 1032.1, 1000.8, 961.5, 837.7, 817.1, 806.1, 784.2, 747.3, 712.9, 637.2. HRMS (+ESI) calculated ($C_{11}H_{15}NO_3$) for 209.2 and observed 248.0 [M^+K^+].

3.3 Hydroxy Bodipy

[[2,2-[[4-[(3,5-dimethyl-1*H*-pyrrol-2-yl- κ*N*)(3,5-dimethyl-2*H*-pyrrol-2-ylidene-κ*N*)methyl]phenyl]imino]bis[ethanolato]](1-)] difluoro-, (*T*-4)-boron (3) 2, 4-dimethylpyrrole (0.43 g, 48 mmol) was combined with compound 2 (0.43 g, 21.6 mmol) in 30 mL DCM. After initial mixing, 4 drops trifluoroacetic acid (TFA) was added and left to stir under nitrogen for 24 h at room temperature. DDQ (0.48 g, 2.13 mmol) was then mixed with DCM (20 mL) and added 1 mL/min to the reaction mixture. The reaction was stirred at room temperature for 24 h and then triethyl amine (8 mL) was added and left to stir at room temperature. After 30 minutes, borontrifluoride ethelate (6.6 mL) was added and the reaction stirred for 24 h at room temperature. The product was extracted using saturated NaCl solution and DCM (3 × 75 mL). The combined extracts were dried over $NaSO_4$. Solvent removal under reduced pressure yielded black oil. The resultant oil was purified using column chromatography with 1:1 hexanes: ether as the eluent and yielded a red solid (536 mg, 27.6 % yield); mp = 148-150 ° C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (d, J = 8.8 Hz, 2 H), 5.98 (s, 2 H), 3.80 (t, J = 7.1 Hz, 4 H), 3.69 (t, J = 6.9

Hz, 4 H), 2.55 (s, 6 H), 1.47(s, 7 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 155.3, 146.9, 143.3, 142.4, 132.3, 129.7, 124.1, 121.3, 112.4, 53.7, 40.5, 29.9, 14.9, 14.8. FT-IR (neat, cm-1) 2963.1, 2927.3, 2907.7, 2870.6, 2359.7, 2324.7, 1981.2, 1726.4, 1606.3, 1539.0, 1506.4, 1470.3, 1444.4, 1406.3, 1354.2, 1305.3, 1265.3, 1237.7, 1187.9, 1156.6, 1124.3, 1085.1, 1049.8, 986.8, 969.7, 919.3, 874.4, 840.8, 813.9, 762.2, 705.0, 657.4, 607.3. HRMS (+ESI) calculated ($C_{13}H_{19}NO_3$) for 237.2 and observed 238.1 [H^+].



Scheme 2: Methoxy Compound Synthesis

3.4 Methoxy Aldehyde

4-[bis(2-methoxyethyl)amino]benzaldehyde (5) N-phenyldiethanolamine (2.0 g, 11 mmol) was added to THF (30 mL), stirring under nitrogen. Sodium hydride (2.2 g, 91 mmol) was added to

the reaction mixture slowly and then refluxed at room temperature for 30 minutes, 40 ° C for 30 minutes and then brought back to room temperature. Methyl iodide (3.43 mL) was added to the reaction mixture slowly and allowed to stir overnight at room temperature. The product (compound 4) was extracted using saturated NaCl solution and DCM (3 × 50 mL). The combined extracts were dried over $NaSO_4$. Solvent removal under reduced pressure yielded yellow oil. The resultant oil was purified using silica column chromatography with 1:1 hexanes: ether as the eluent and yielded a clear oil. This compound was then used as a starting material for the aldehyde. Compound 4 (0.15 g, 0.72 mmol) was added to DMF (4 mL). Phosphorus oxychloride (0.55 g, 3.59 mmol) was added to the reaction mixture slowly. After 24 h in a 60 ° C oil bath, the reaction mixture was applied over ice and neutralized to pH 7 with saturated $NaHCO_3$. The product was extracted using saturated NaCl solution and ethyl acetate (3 × 50 mL). The combined extracts were dried over $NaSO_4$. Solvent removal under reduced pressure yielded brown oil. The resultant oil was purified using column chromatography with 1:1 hexanes: ether as the eluent and yielded a yellow oil (0.39 g, 24.4 % yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.73 (s, 1 H), 7.71 (d, J = 9.3 Hz, 2 H), 6.75 (d, J = 9.4 Hz, 2 H), 3.66 (t, J = 5.9 Hz, 4 H), 3.58 (t, J = 5.8 Hz, 4 H), 3.35 (s, 6 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 190.2, 152.8, 132.3, 125.5, 111.2, 70.0, 59.2, 51.2. FT-IR (neat, cm-1) 2924.9, 2879.0, 2812.6, 2733.9, 2152.8, 2028.4, 1666.8, 1590.2, 1555.8, 1522.7, 1452.4, 1434.1, 1399.7, 1356.7, 1314.3, 1279.1, 1237.9, 1197.3, 1165.0, 1108.0, 1009.9, 1000.1, 959.7, 908.9, 813.8, 729.8, 710.4. HRMS (+ESI) calculated ($C_{11}H_{15}NO_3$) for 209.2 and observed 248.0 [M^+K^+].

3.5 Methoxy Bodipy

[[2,2-[[4-[(3,5-dimethyl-1*H*-pyrrol-2-yl- κ*N*)(3,5-dimethyl-2*H*-pyrrol-2-ylidene-κ*N*)methyl] phenyl]imino]bis[methoxyethanolato]](1-)] difluoro-, (*T*-4)-boron (6) 2,4-dimethylpyrrole

(0.34 g, 3.58 mmol) was added to compound 4 (0.39 g, 1.63 mmol) in DCM (25 mL). After initial mixing, 3 drops trifluoroacetic acid was added and left to stir under nitrogen for 24 h at room temperature. DDQ (0.38 g, 1.68 mmol) was then mixed with DCM (20 mL) and added 1 mL/min to the reaction mixture. The reaction was stirred at room temperature for 24 h and then triethyl amine (6 mL) was added and left to stir at room temperature. After 30 minutes, borontrifluoride ethelate (2.98 g, 21 mmol) was added and the reaction stirred for 24 h at room temperature. The product was extracted using saturated NaCl solution and DCM (3 × 75 mL). The combined extracts were dried over $NaSO_4$. Solvent removal under reduced pressure yielded black oil. The resultant oil was purified using silica column chromatography with 1:1 hexanes: ether as the eluent and yielded a red solid (trace yield); mp = 110-114 ° C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07 (d, J = 8.9 Hz, 2 H), 8.86 (d, J = 8.9 Hz, 2 H), 6.07 (s, 2 H), 3.56 (m, 8 H), 3.31 (s, 6 H). ^{13}C NMR (500 MHz, $CDCl_3$) δ 154.9, 148.7, 143.4, 132.4, 129.2, 122.4, 121.1, 112.4, 70.2, 59.3, 51.2, 29.9, 14.9. FT-IR (neat, cm-1) 3098.7, 2957.7, 2923.0, 2884.1, 2824.9, 2738.0, 2339.4, 1727.5, 1668.4, 1606.8, 1540.3, 1525.8, 1499.8, 1468.4, 1396.0, 1359.9, 1305.6, 1295.1, 1265.5, 1232.7, 1188.2, 1156.9, 1106.4, 1061.8, 1009.3, 970.1, 902.2, 813.7, 761.8, 703.8, 647.8. HRMS (+ESI) calculated ($BC_{25}F_2H_{32}N_3O_2$) for 455.3 and observed 455.3 and 456.2 [H^+].

Conclusions & Future Research

This research resulted in the synthesis and characterization of two BODIPY sensors which do not have a strong binding affinity for iron. To further this research, these sensors should be studied using UV-vis, titration experiments with iron, and fluorescence studies. Additionally, these sensors should be compared side-by-side with another published BODIPY sensor which is known to bind to iron in both organic solvent and aqueous solvent to demonstrate differences and similarities in fluorescence response. Finally, these sensors should

be used in cells and cellular images should be taken which show florescence under varying sensor and Fe^{3+} conditions. If the sensors created in this MQP, which should not bind iron, show similar fluorescence responses when compared to the BODIPY sensors which should bind iron, that may provide evidence that iron binding is not necessary to induce a fluorescence response.

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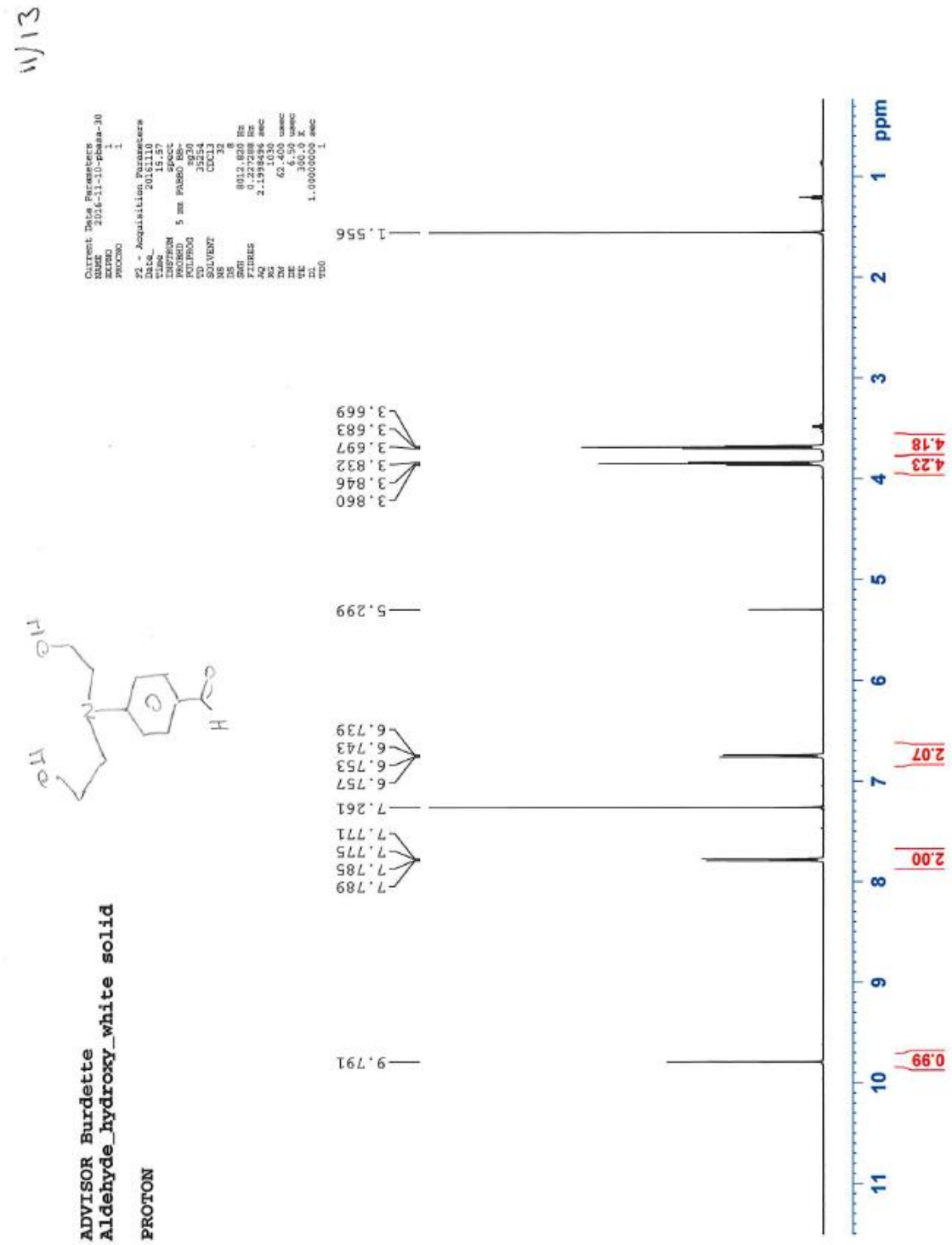
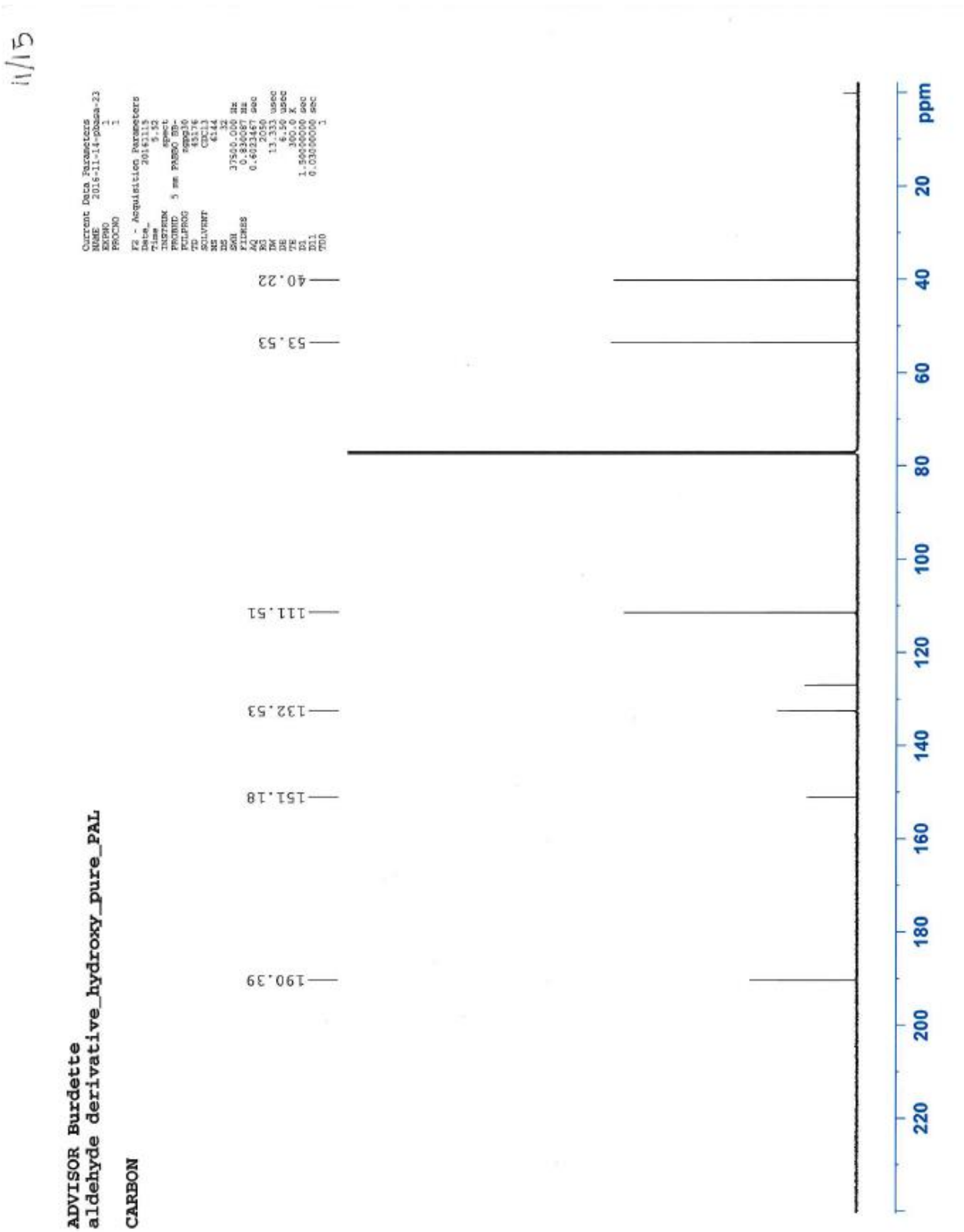
Supplementary Information

Supplementary Figure 1: Hydroxy Aldehyde ^{1}H NMR



Supplementary Figure 2: Hydroxy Aldehyde ^{13}C NMR



Supplementary Figure 3: Hydroxy Aldehyde LC-MS

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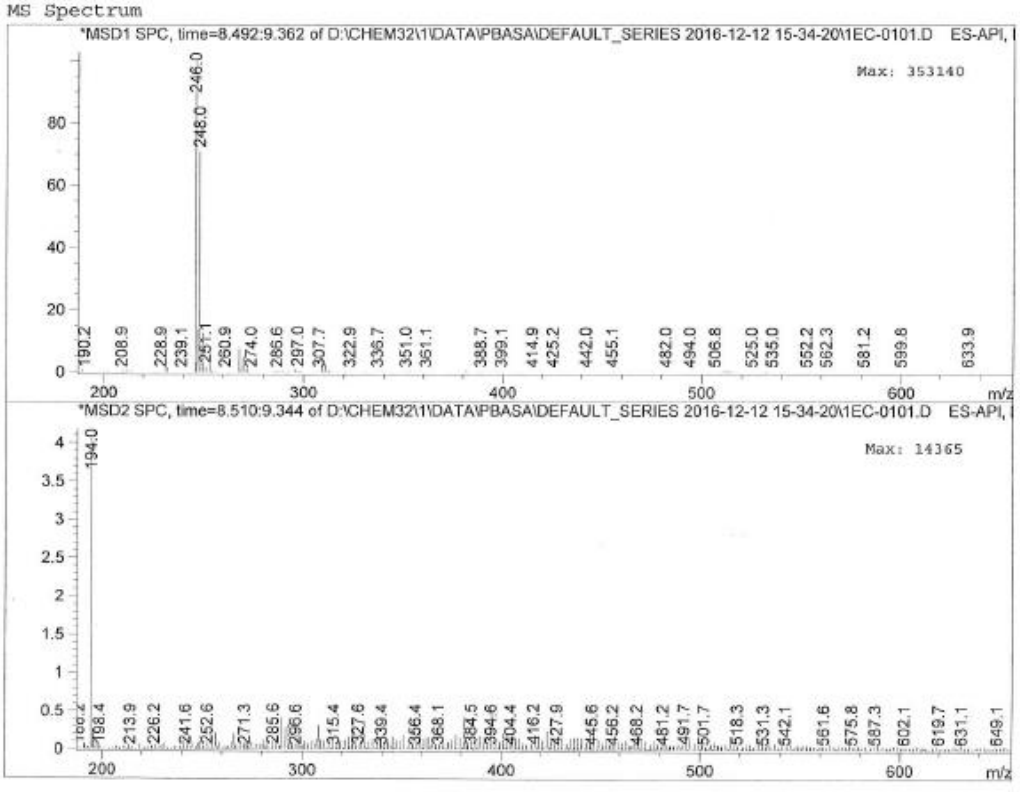
Sample Name : Aldehyde_derivative_PL_repeat

Acq. Operator : PBASA Seq. Line : 1
Acq. Instrument : Instrument 1 Location : P1-E-03
Injection Date : 12/12/2016 3:34:55 PM Inj : 1
Inj Volume : 2 µl

Method : D:\CHEM32\1\DATA\PBASA\DEFAULT_SERIES 2016-12-12 15-34-20\ACID_GRAD_SERIES.M
Last changed : 6/29/2016 10:12:56 AM by DRJOHNSON
Method Info : Method: APB_ACID_GRAD_SERIES.M, multi-sample, 18 minutes run time/sample
2uL injection with 10sec Needle Wash (flushport) for LC-MS, ESI+/-, m/z 180-1200
Use with Sequence DEFAULT_SERIES.S, tune file: atunes_dual_fast.TUN
A1 H2O 0.1% FA, B1 95%ACN/5%H2O w0.1%FA,
0.3ml/Min gradient, 8 minutes, 30oC, Column 1
*For Use with ES Ind. Epic C18 MSO, 2.3u, 150A, 5cmx2.1mm column, S/N: 298-13-80253, Max Pressure Limit 350bar, 06/19/14, APB
*For Use with G1367B Autosampler, 08/15/13, APB

Sample Info : 1mM in DMF and dilute in methanol

MS Spectrum



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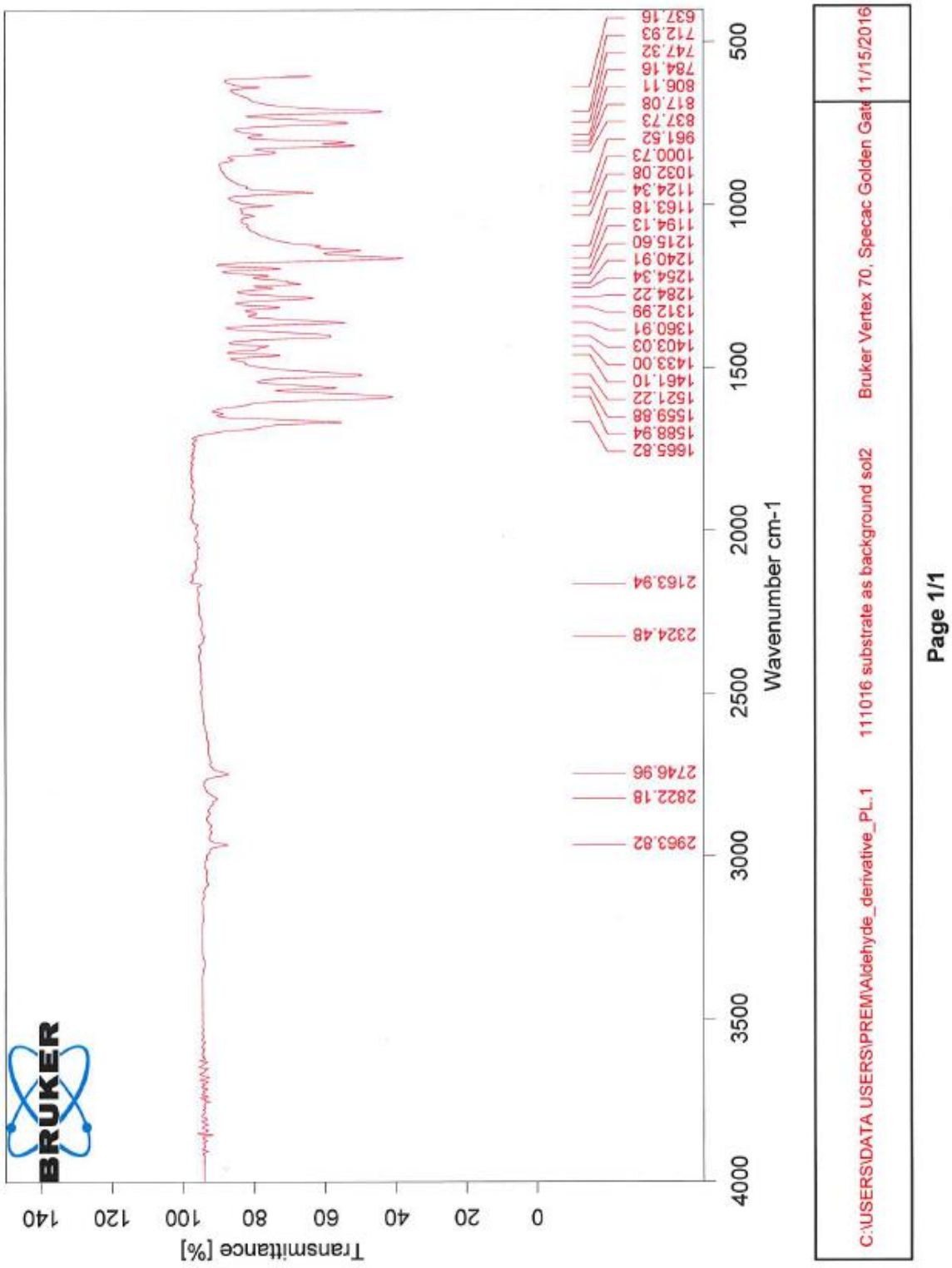
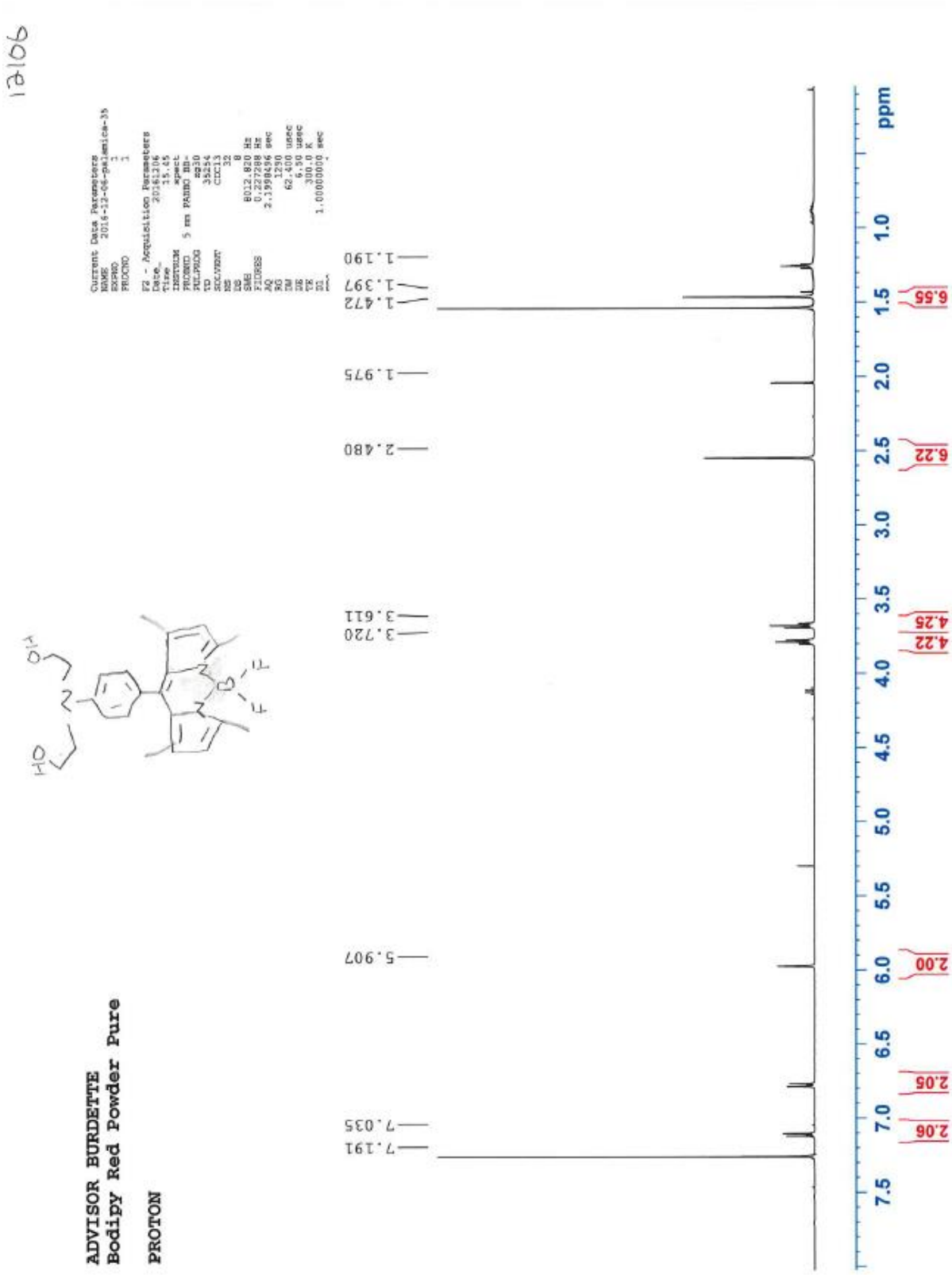
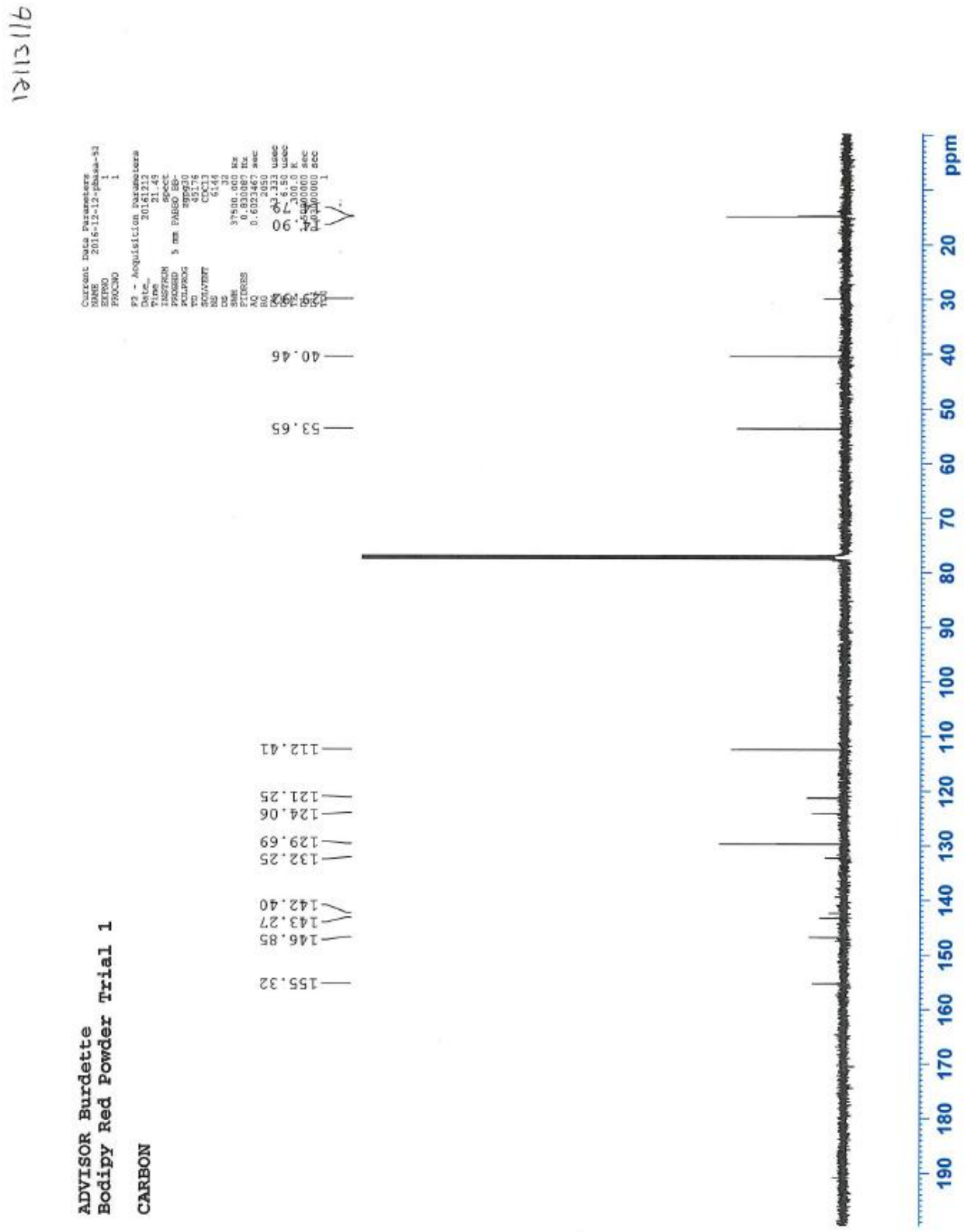
Supplementary Figure 4: Hydroxy Aldehyde Transmittance



Supplementary Figure 5: Hydroxy BODIPY ^{1}H NMR



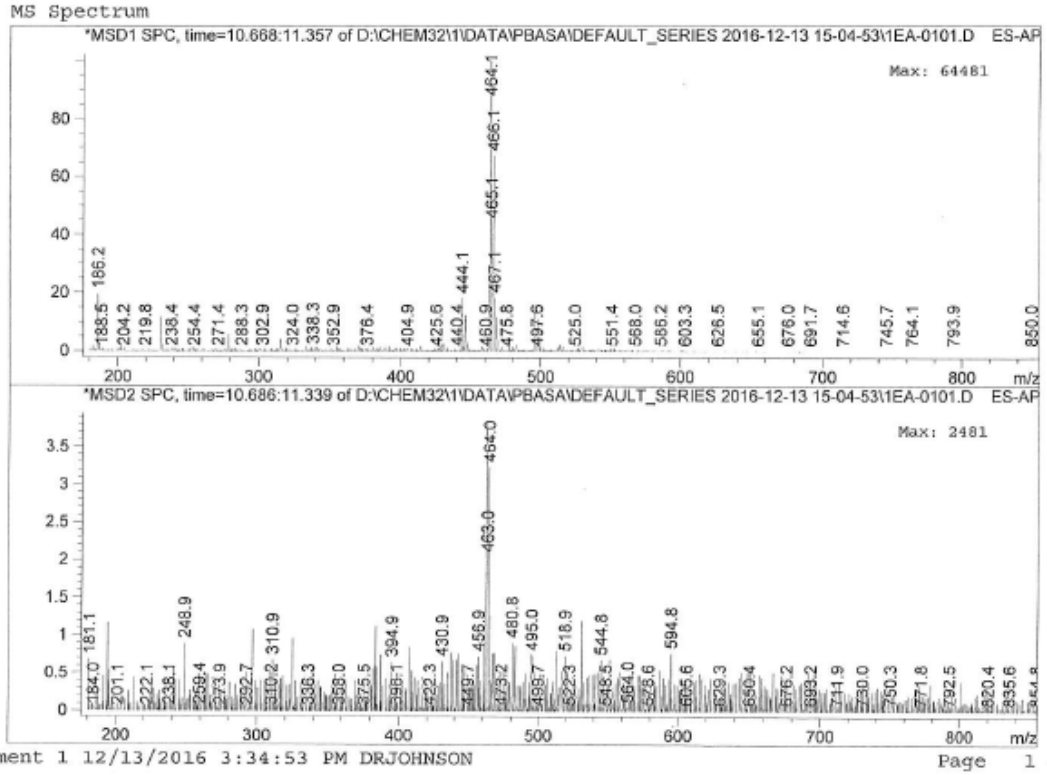
Supplementary Figure 6: Hydroxy BODIPY ^{13}C NMR



Supplementary Figure 7: Hydroxy BODIPY LC-MS

```
Print of window 80: MS Spectrum
Data File   : D:\CHEM32\1\DATA\PBASA\DEFAULT_SERIES 2016-12-13 15-04-53\1EA-0101.D
Sample Name : Bodipy_Repeat
=====================================================================
Acq. Operator    : PBASA                          Seq. Line :   1
Acq. Instrument  : Instrument 1                    Location : P1-E-01
Injection Date   : 12/13/2016 3:05:25 PM                Inj :   1
                                                 Inj Volume : 2 µl
Acq. Method      : D:\CHEM32\1\DATA\PBASA\DEFAULT_SERIES 2016-12-13 15-04-53\ACID_GRAD_
                   SERIES.M
Last changed     : 6/29/2016 10:12:56 AM by DRJOHNSON
Analysis Method  : D:\CHEM32\1\DATA\PBASA\DEFAULT_SERIES 2016-12-13 15-04-53\ACID_GRAD_
                   SERIES.M
Last changed     : 12/13/2016 3:31:32 PM by DRJOHNSON
Method Info      : Method: APB_ACID_GRAD_SERIES.M, multi-sample, 18 minutes run time/
                   sample
                   2uL injection with 10sec Needle Wash (flushport) for LC-MS, ESI+/-,
                   m/z 180-1200
                   Use with Sequence DEFAULT_SERIES.S, tune file: atunes_dual_fast.TUN
                   A1 H2O 0.1% FA, B1 95%ACN/5%H2O w0.1%FA,
                   0.3ml/Min gradient, 8 minutes, 30oC, Column 1
                   *For Use with ES Ind. Epic C18 MSO, 2.3u, 150A, 5cmx2.1mm column, S/
                   N: 298-13-80253, Max Pressure Limit 350bar, 06/19/14, APB
                   *For Use with G1367B Autosampler, 08/15/13, APB


Sample Info      : 1mM in DMF and dilute in methanol
```



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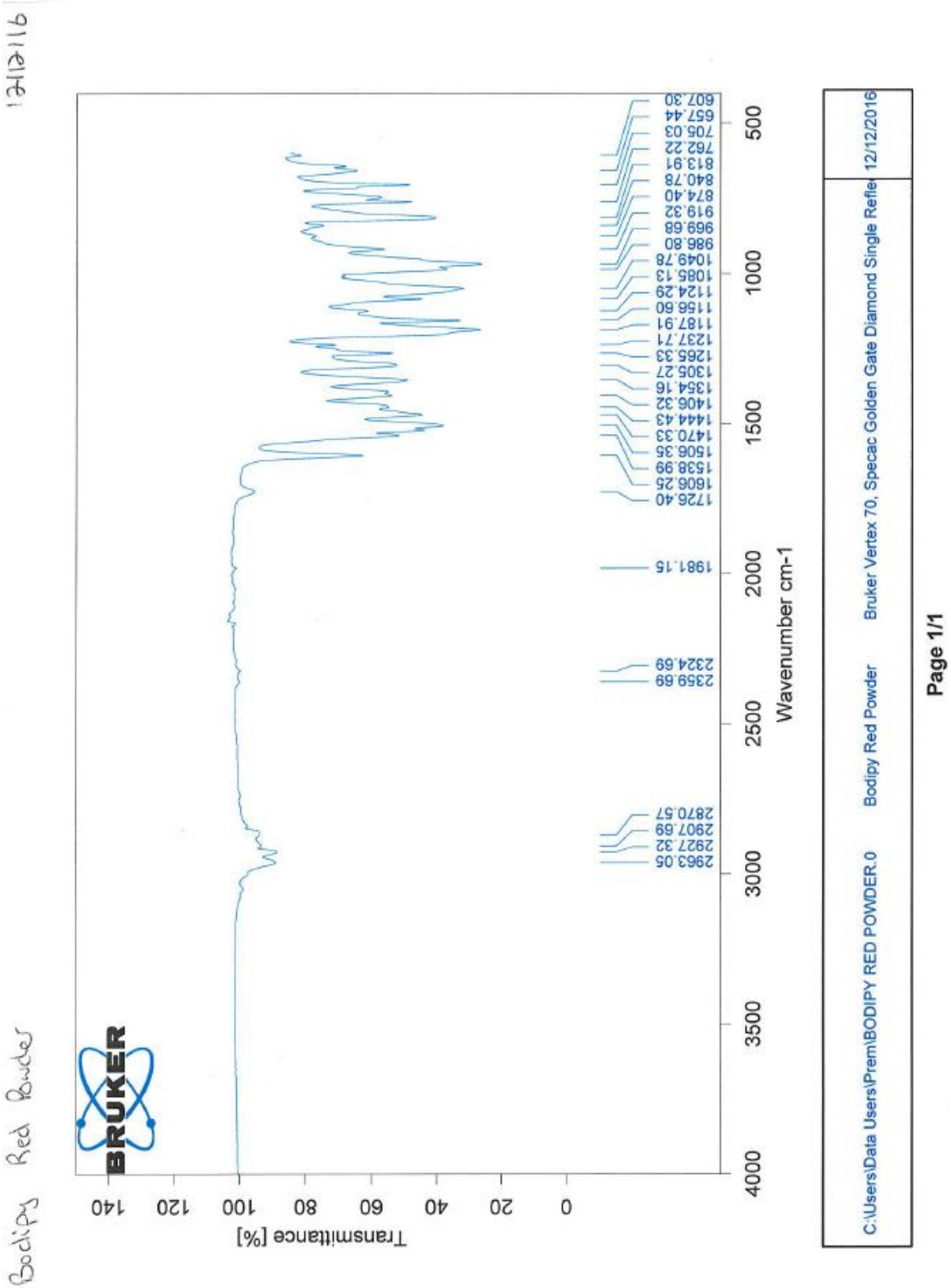
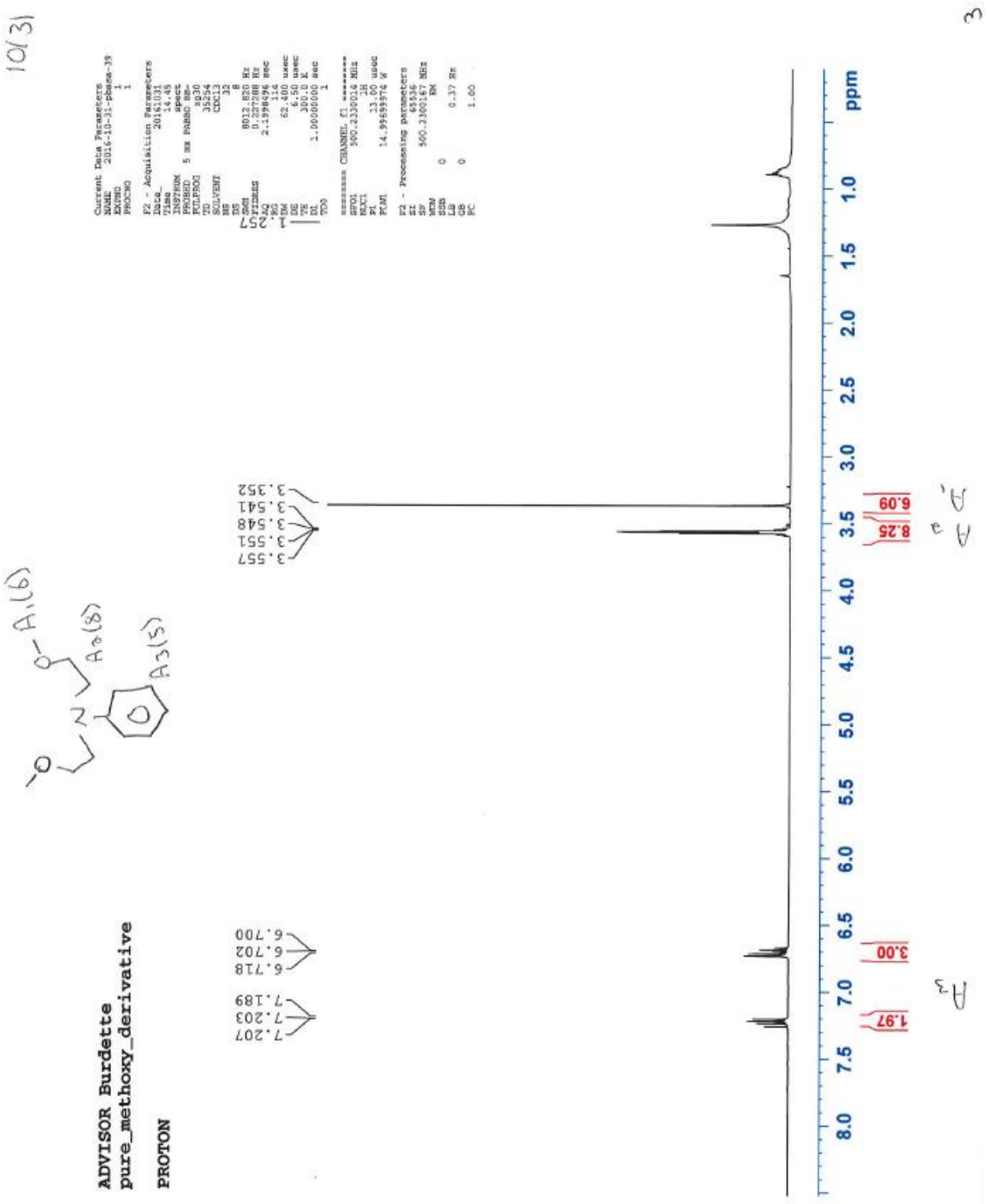
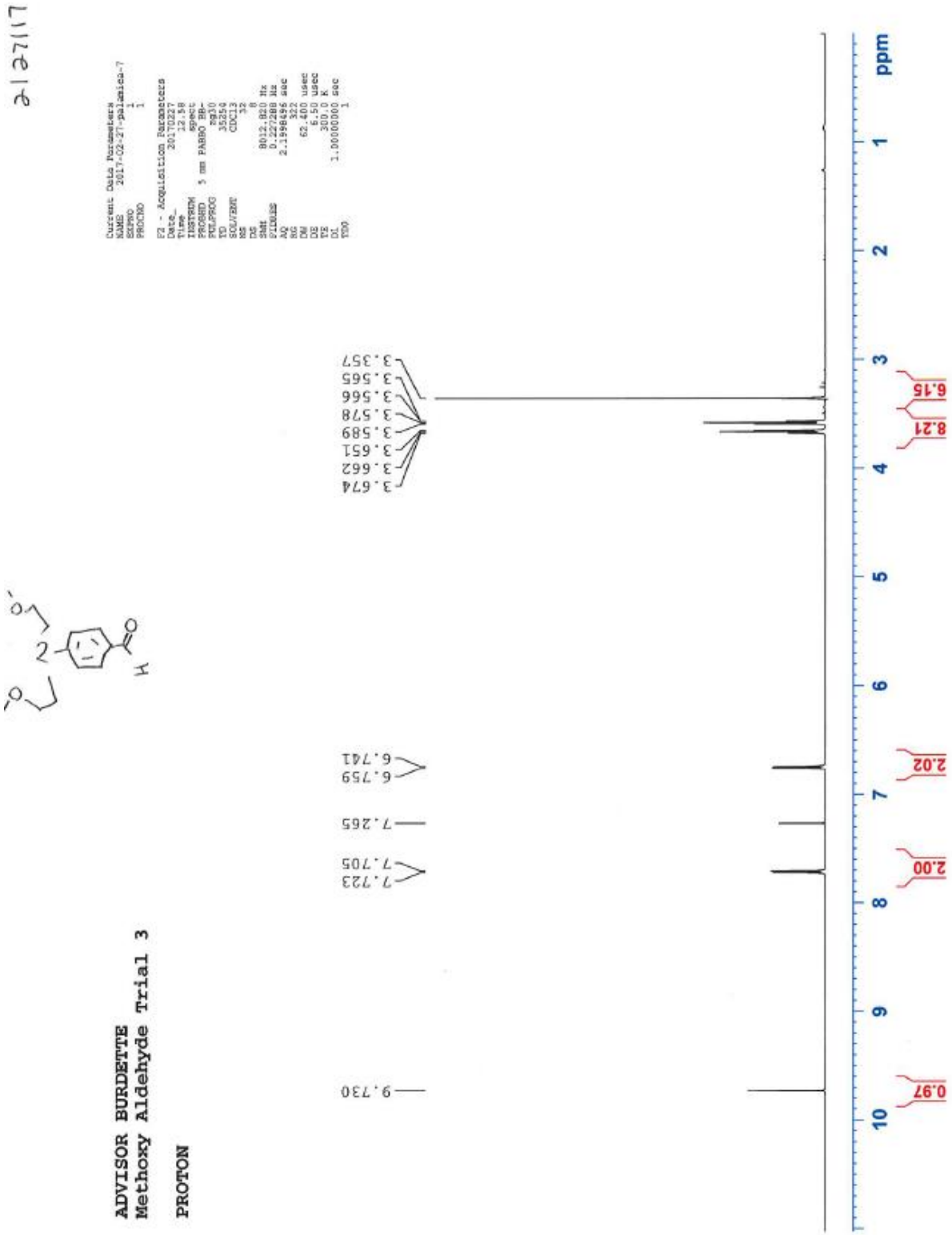
Supplementary Figure 8: Hydroxy BODIPY Transmittance



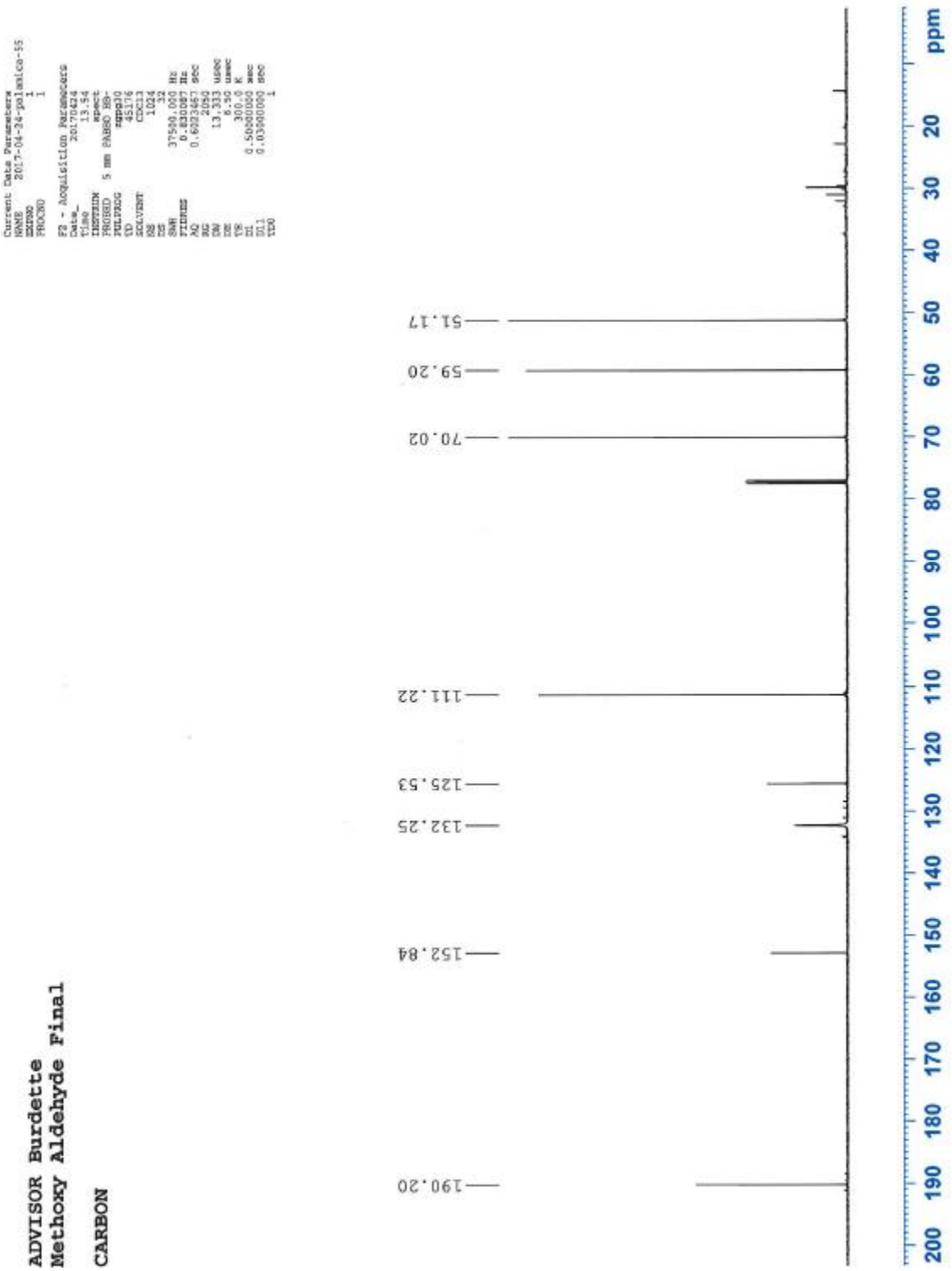
Supplementary Figure 9: Methoxy Derivative ^{1}H NMR



Supplementary Figure 10: Methoxy Aldehyde ^{1}H NMR

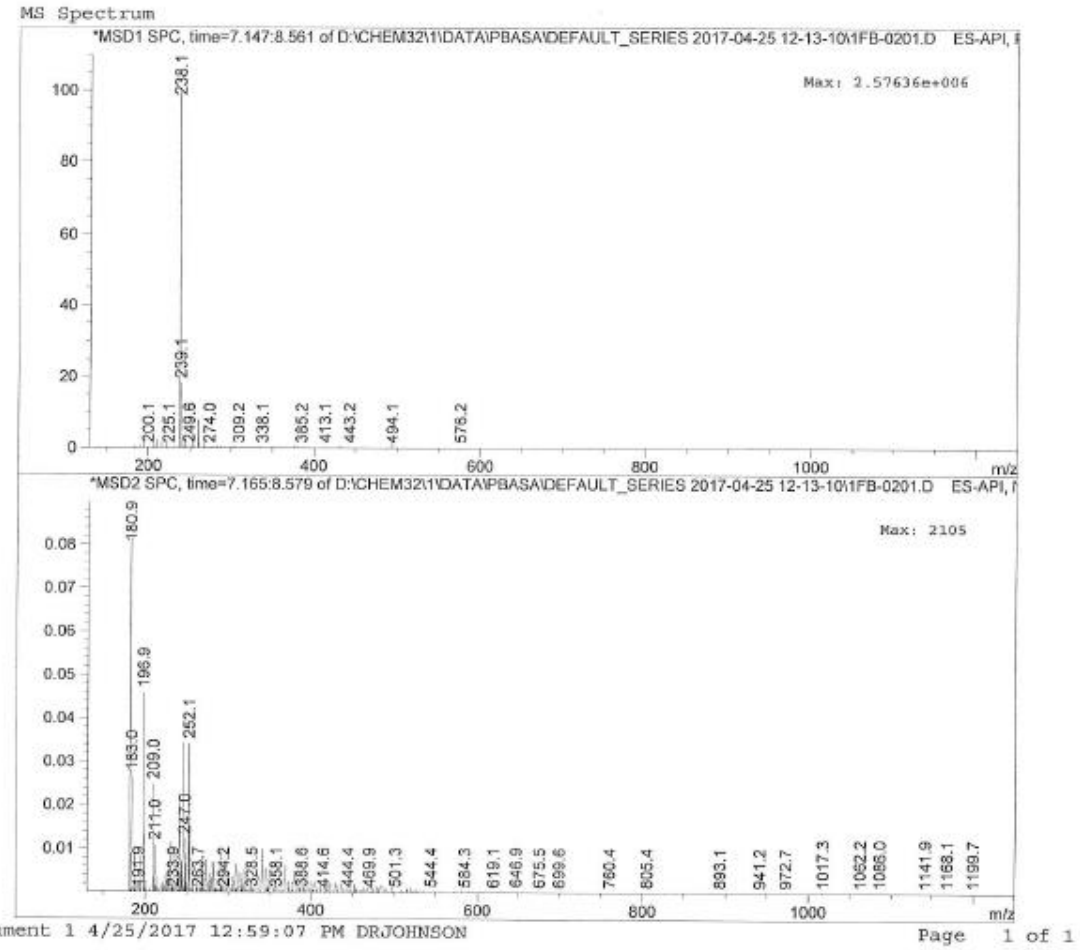


Supplementary Figure 11: Methoxy Aldehyde ^{13}C NMR



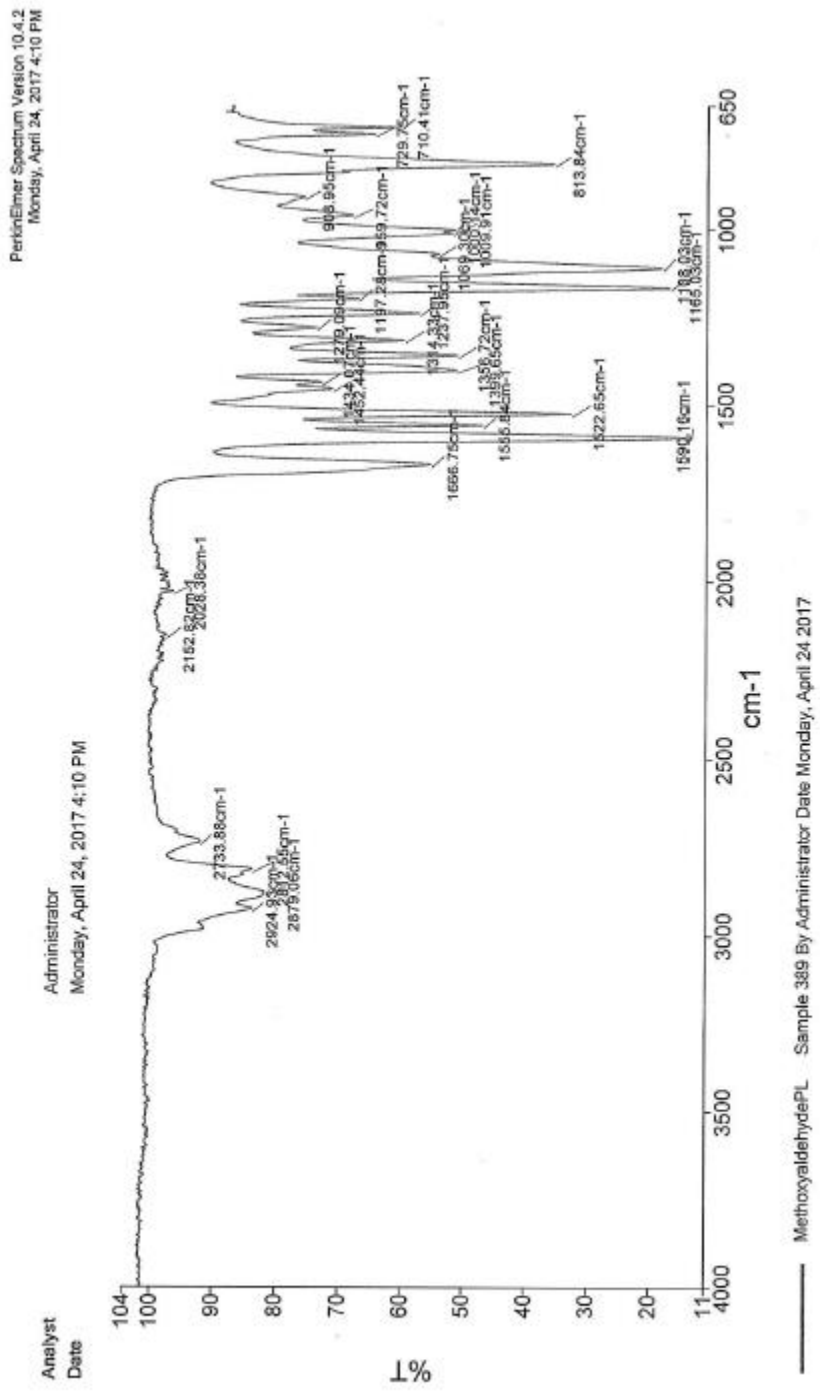
Supplementary Figure 12: Methoxy Aldehyde LC-MS

```
Print of window 80: MS Spectrum
Data File   : D:\CHEM32\1\DATA\PBASA\DEFAULT_SERIES 2017-04-25 12-13-10\1FB-0201.D
Sample Name : Methoxy Alehyde PL
=====================================================================
Acq. Operator    : PBASA                          Seq. Line :   2
Acq. Instrument  : Instrument 1                    Location : P1-F-02
Injection Date   : 4/25/2017 12:29:22 PM               Inj :   1
                                                  Inj Volume : 2 µl
Method           : D:\CHEM32\1\DATA\PBASA\DEFAULT_SERIES 2017-04-25 12-13-10\ACID_GRAD_
                   SERIES.M
Last changed     : 2/21/2017 1:42:21 PM by SAINT-GOBAIN
Method Info      : Method: APB_ACID_GRAD_SERIES.M, multi-sample, 18 minutes run time/
                   sample
                   2uL injection with 10sec Needle Wash (flushport) for LC-MS, ESI+/-,
                   m/z 180-1200
                   Use with Sequence DEFAULT_SERIES.S, tune file: atunes_dual_fast.TUN
                   A1 H2O 0.1% FA, B1 95%ACN/5%H2O w0.1%FA,
                   0.3ml/Min gradient, 8 minutes, 30oC, Column 1
                   *For Use with ES Ind. Epic C18 MSO, 2.3u, 150A, 5cmx2.1mm column, S/
                   N: 298-13-80253, Max Pressure Limit 350bar, 06/19/14, APB
                   *For Use with G1367B Autosampler, 08/15/13, APB
```

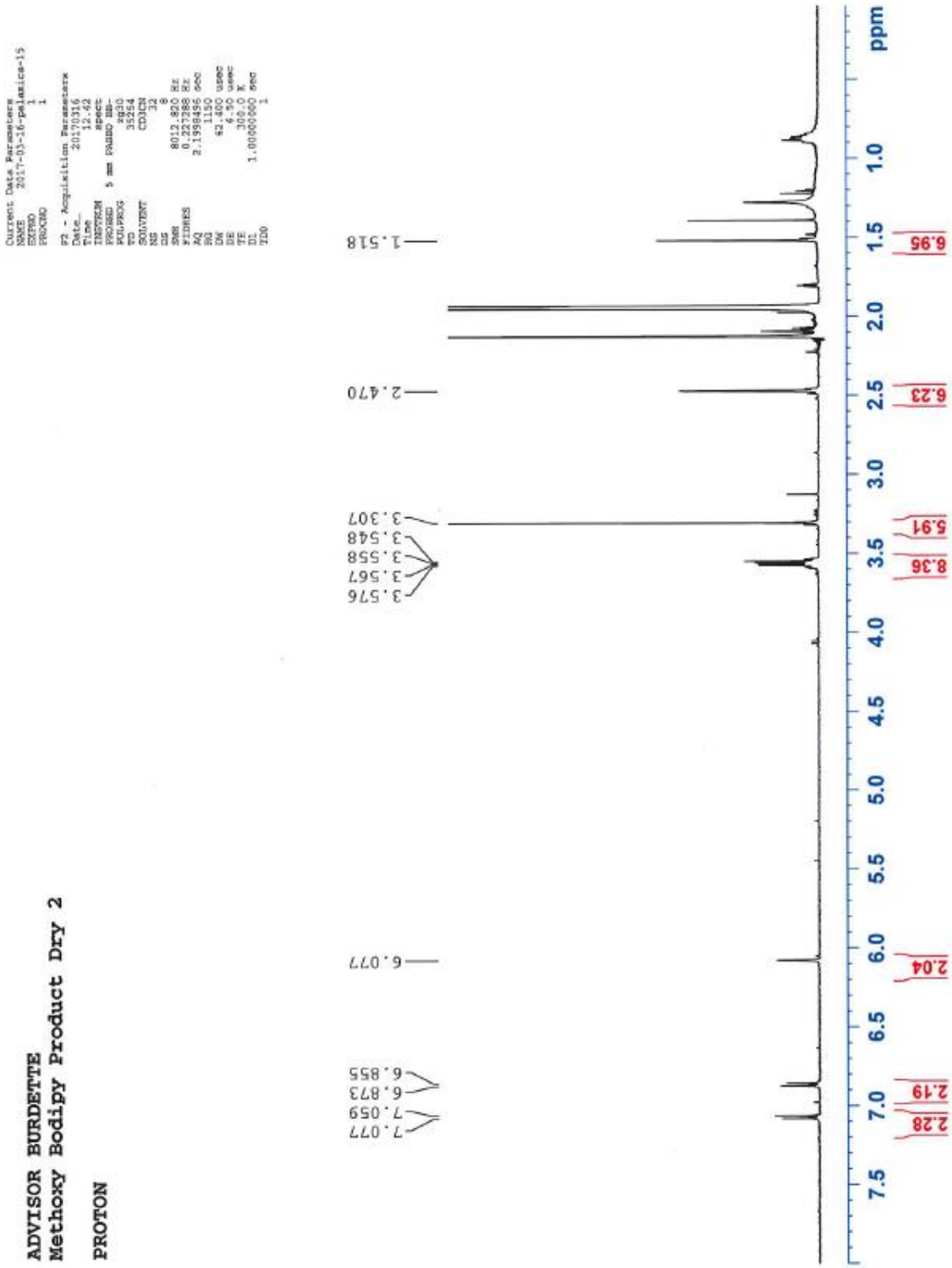


Instrument 1 4/25/2017 12:59:07 PM DRJOHNSON Page 1 of 1

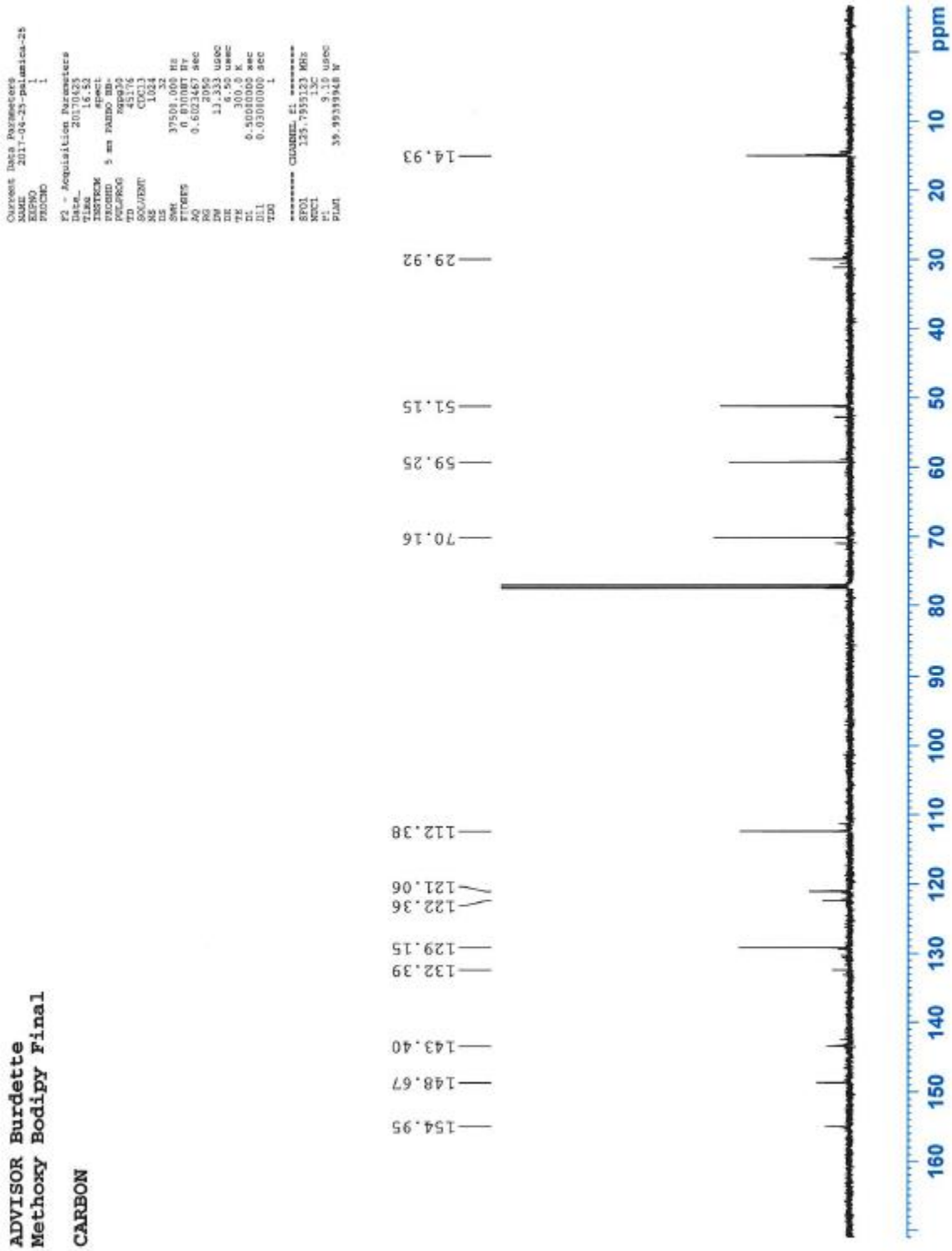
Supplementary Figure 13: Methoxy Aldehyde Transmittance



Supplementary Figure 14: Methoxy BODIPY ^{1}H NMR

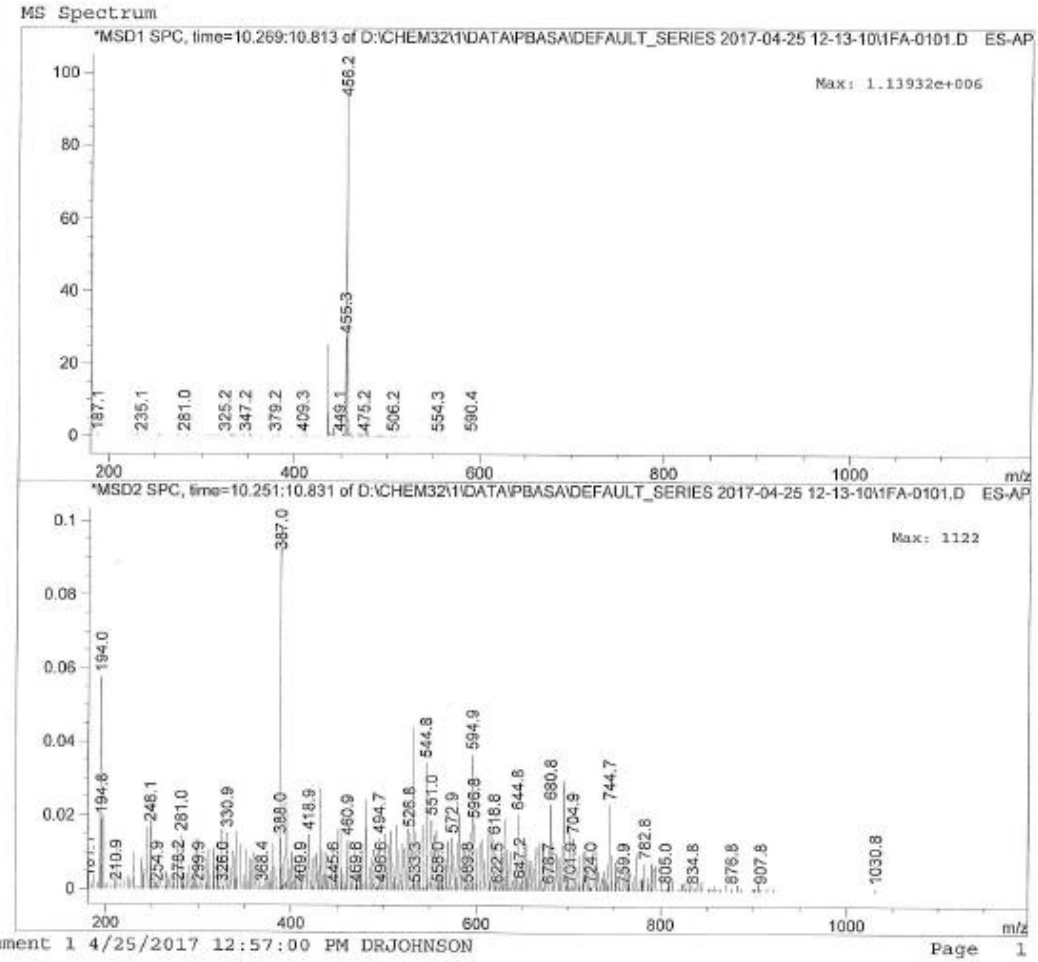


Supplementary Figure 14: Methoxy BODIPY ^{13}C NMR



Supplementary Figure 15: Methoxy BODIPY LC-MS

```
Print of window 80: MS Spectrum
Data File  : D:\CHEM32\1\DATA\PBASA\DEFAULT_SERIES 2017-04-25 12-13-10\1FA-0101.D
Sample Name : Methoxy Bodipy PL
======================================================================
Acq. Operator   : PBASA                          Seq. Line :   1
Acq. Instrument : Instrument 1                    Location : P1-F-01
Injection Date  : 4/25/2017 12:13:52 PM              Inj :   1
                                                 Inj Volume : 2 µl
Method          : D:\CHEM32\1\DATA\PBASA\DEFAULT_SERIES 2017-04-25 12-13-10\ACID_GRAD_
                  SERIES.M
Last changed    : 2/21/2017 1:42:21 PM by SAINT-GOBAIN
Method Info     : Method: APB_ACID_GRAD_SERIES.M, multi-sample, 18 minutes run time/
                  sample
                  2uL injection with 10sec Needle Wash (flushport) for LC-MS, ESI+/-,
                  m/z 180-1200
                  Use with Sequence DEFAULT_SERIES.S, tune file: atunes_dual_fast.TUN
                  A1 H2O 0.1% FA, B1 95%ACN/5%H2O w0.1%FA,
                  0.3ml/Min gradient, 8 minutes, 30oC, Column 1
                  *For Use with ES Ind. Epic C18 MSO, 2.3u, 150A, 5cmx2.1mm column, S/
                  N: 298-13-80253, Max Pressure Limit 350bar, 06/19/14, APB
                  *For Use with G1367B Autosampler, 08/15/13, APB
```



Supplementary Figure 16: Methoxy BODIPY Transmittance